rBGA

Version 23_EN_RUO, 2023-07

RUO	For research use only
Σ	PU 300 µl: 75 – 150 PU.50 µl: 12 – 25
2°C 8°C	2...8°C
	See package printings

1. Introduction

1.1. Overview

This manual describes the protocol for the imusyn

recombinant blood group antigens (rBGA)

for the specification of irregular anti-erythrocytic antibodies.

The detection of anti-erythrocytic antibodies in patients is a central diagnostic requirement in pre- and post-blood transfusion investigations. Screening for irregular anti-erythrocytic antibodies is mandatory for all patients on blood transfusion in Germany[1]. Here, about 10% of all hospital in-patients per year need a blood transfusion and up to 4% of these patients are positive for irregular anti-erythrocytic antibodies.

However, in some patients a clear identification of anti-erythrocytic antibodies specificities can be difficult, and sometimes even impossible. This is true in patients with a mixture of different anti-erythrocytic antibodies, in patients with anti-erythrocytic autoantibodies and, in particular, in patients with antibodies against high frequency erythrocytic antigens[2]. Clinically relevant antibodies can be masked by clinically irrelevant antibodies and thereby hamper pre-transfusional diagnostics. The imusyn rBGA are designed to inhibit anti-erythrocytic antibodies[3], thereby facilitating anti-erythrocytic antibody identification and minimizing the risk of selecting incompatible erythrocyte concentrates for patients.

1.2. Test Principle

rBGA work as inhibiting molecules. The sample is incubated with rBGAs prior to an indirect antiglobulin test (IAT) to neutralize specific antibodies to the according BGAs. It can then be investigated in the IAT for the presence of other antibodies without any further treatment.

1.3. Statement of Intended Use

For research use only.

Identification and confirmation of irregular anti-erythrocytic antibodies. rBGAs are for use with gel card systems (Grifols DG Gel, Bio-Rad ID-System, ORTHO BioVue, ORTHO MTS, or Cellbind Screen) or in tube testing.

2. Materials and Equipment

2.1. Definition of Symbols

PBS	Phosphate buffered saline pH 7.4
rBGA	recombinant blood group antigen
NC	Negative control
NaCl	0,9 % sodium chloride solution

2.2. Components

Recombinant blood group antigens rBGA 300 µl/50 µl, concentration: 0.5 mg/ml. Conserved with 0.1% ProClin® 300

WARNING!

May cause an allergic skin reaction (H317). Harmful to aquatic life with long lasting effects (H412). Wear protective gloves (P280). If skin irritation or rash occurs: Get medical advice/attention (P333+P313). Dispose of contents/container in accordance with local/regional/national/international regulations (P501).

REF	Antigen
R_Ch(a)	**C4B*3**
R_CR1_2	**Kn(a)**, McC(a), Sl(a), Sl3+, KCAM+, Yk(a), **DACY**
R_CROM	Cr(a+), Tc(a), **Dr(a+)**, Es(a+), IFC+, WES(b), UMC+, GUTI+, SERF+, CROZ+, CROV+, ZENA+, CRAM+, CROK+, CORS+, CRUE+, CRAG+
R_Do(a)	**Do(a)**, Hy+, Jo(a+), DOLG+, DOYA+, DOMR+, DOLC+, DODE+
R_Do(b)	**Do(b)**, Hy+, Jo(a+), DOLG+, DOYA+, DOMR+, DOLC+, DODE+
R_Fy(a)	**Fy(a)**, Fy6
R_Fy(b)	**Fy(b)**, Fy6
R_grKba	Js(a), K12+, Ul(a-), K19+, TOU+, K23-, K13+, K22+, K11, **Kp(b)**, RAZ+, VLAN+, **K**, K14/24, K18+, KASH+, KELP+, KYO-, KHUL+, KTIM+, KUCI+, KANT+, KETI+, KALT+, VONG+
R_In(b)	**In(b)**, INFI+, INJA+, INRA+, INSL+
R_JMH	**JMH1,** JMH2, JMH3, JMH4, JMH5, JMH6, JMH7, JMH8
R_klkba	Js(a), K12+, Ul(a-), K19+, TOU+, K23-, K13+, K22+, K11, **Kp(b)**, RAZ+, VLAN+, **k**, K14/24, K18+, KASH+, KELP+, KYO-, KHUL+, KTIM+, KUCI+, KANT+, KETI+, KALT+, VONG+
R_Lu(a)	**Lu(a)**, Lu4+, Lu5+, Lu6, Lu8, Lu12+, Lu13+, Lu16+, Lu17+, Lu20+, Lu21+, LURC+, Lu7+, Lu23, Lu24, Lu25, Lu27, LU28, LU29
R_Lu(a)_2	**Lu(a)**, Lu4+, Lu5+, Lu6, Lu8, Lu12+, Lu13+, Lu16+, Lu17+, Lu20+, Lu21+, LURC+, Lu7+, Lu23, Lu24, Lu25, Lu27, Lu18, LU28, LU29
R_Lu(b)	**Lu(b)**, Lu4+, Lu5+, Lu6, Lu8, Lu12+, Lu13+, Lu16+, Lu17+, Lu20+, Lu21+, LURC+, Lu7+, Lu23, Lu24, Lu25, Lu27, LU28, LU29
R_Lu(b)_2	**Lu(b)**, Lu4+, Lu5+, Lu6, Lu8, Lu12+, Lu13+, Lu16+, Lu17+, Lu20+, Lu21+, LURC+, Lu7+, Lu23, Lu24, Lu25, Lu27, **Lu19**, LU28, LU29
R_LW(a)	**LW(a), LW6, LW8**
R_Rg(a)	**C4A*3**
R_Sc1	**Sc1**, Rd-, SCAN+, STAR+, SCER+, SCAR+, SCAC+
R_Xg(a)	**Xg(a)**
R_YCAD	**YCAD**
R_Yt(a)	**Yt(a),** YTEG+, YTLI+, YTOT+, YTGT+

Table 1) List of rBGA with corresponding antigens. Serologically tested antigens are in bold.

2.3. Storage and Expiry Date

Store at 2…8°C. The expiry date is given on the label of the immediate container. If the storage conditions are met, the proteins can be used until the expiry date given on the immediate container and the certificate of analysis.

WARNING!

Do not freeze rBGA! The reactivity of frozen or frozen and thawed proteins cannot be guaranteed. The according proteins have to be disposed of immediately!

2.4. Materials and Equipment Supplied by the User

The following materials and equipment have to be provided by the user:

2.4.1. Materials

Material	Supplier
Gel cards: DG Gel Coombs, ID-Card LISS/Coombs, ORTHO BioVue, ORTHO MTS, or Cellbind Screen	Grifols, Bio-Rad, Ortho Diagnostics, Sanquin
Test cell reagents for the according gel card system	Grifols, Bio-Rad, Ortho Diagnostics, Sanquin
Alternatively: materials and test cells for tube testing	Multiple suppliers
Polypropylene reaction tube	Multiple suppliers
PBS Phosphate buffered saline, pH 7.4 or NaCl 0.9% sodium chloride solution	Multiple suppliers
Pipette tips	Multiple suppliers

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2.4.2. Equipment

Equipment	Supplier
Gel card centrifuge or work station, matching the gel card system used	Grifols, Bio-Rad, Ortho Diagnostics, Sanquin
Alternatively: centrifuge for tube testing	Multiple suppliers
Incubator, 37°C	Multiple suppliers
Pipettes	Multiple suppliers
Tabletop centrifuge	Multiple suppliers

Note: The reagents and instruments listed above with a specific supplier given have been validated for the use with rBGAs. Reagent and instruments other than those must be validated by the user before use.

3. Preparation and Usage

Contaminations have to be avoided during all steps.

⚠ WARNING! Only use rBGA that come in undamaged containers! All components have to be disposed of according to local regulations.

3.1. Sample Preparation

Serum or plasma samples have to be fresh and neither hemolytic nor lipaemic. Preferably, blood samples should be drawn into citrate, EDTA or CPD-A anticoagulant. Particles, aggregates, or fibrin residues have to be removed prior to testing to avoid unspecific reactions. Samples should be stored at 2..8°C for no longer than 48 hrs. Also follow the restrictions for samples given by the manufacturer of your gel card system.

⚠ WARNING! Human samples pose a potential health hazard. Treat the samples as potentially infectious and take according protective measures as stated in your local guidelines.

3.2. Test Procedure

Pre-incubation of rBGA and sample in a test tube

Test system	sample-rBGA-solution	NC
Tube test	4 µl rBGA + 50 µl sample	4 µl PBS or NaCl + 50 µl sample
Ortho BioVue	3,5 µl rBGA + 40 µl sample	3,5 µl PBS or NaCl + 40 µl sample
others	2 µl rBGA + 25 µl sample	2 µl PBS or NaCl + 25 µl sample

Prepare the sample-rBGA-solution and the NC according to the table above, depending on the test system you intend to use. Vortex both mixtures briefly for 5 sec. Spin the liquid down in a table-top centrifuge for 5 sec at 8000 x g.

Incubate for 30 min at room temperature (19-25°C).

Indirect antiglobulin test

Analyse the sample-rBGA-solution and the NC in a suitable test system (see manufacturer manual).

4. Analysis and Troubleshooting

4.1. Analysis

The test results should always be interpreted in respect to other data of the sample. The result of this assay should not be used as the sole basis for a finding.

Positive:

A sample is positive for antibodies against the used rBGA if the reaction (agglutination) of the sample with rBGA on antigen-positive red cells is negative in comparison with the NC.

Negative:

A sample is negative for antibodies against the used rBGA if the strength of the reaction of the sample with rBGA on antigen-positive red cells is not reduced in comparison with the NC.

Another antibody might be present in the sample if the strength of the reaction is reduced but the agglutination is not completely inhibited. If the NC does not agglutinate, the result cannot be used for evaluation.

The guidelines for interpreting the strength of a reaction can be found in the manual of the gel card manufacturer.

4.2. Troubleshooting

Problem	Possible Cause	Solution
Incomplete inhibition of the serum.	Antibodies with high titer Antibodies with high avidity	Increase the amount of protein step by step. Dilute the sample (Note that additional antibodies may drop below detection level.)
	The sample-rBGA-mix has not been sufficiently mixed (critical step).	Repeat the test and mix the samples according to the manual.

The functionality of rBGA has to be checked with a reference sample. Please also consider the notes of the gel card manufacturer on troubleshooting and limitations. It is recommended to titrate all samples to identify samples with high titer.

5. Limitations and Specific Characterization

5.1. Limitations

Incorrect execution of the instructions given in this manual can lead to false results. In particular, wrong incubation times and temperatures as well as failure to mix rBGA and sample may lead to false negative results.

Contamination of reagents or samples, usage of reagents over their life-time, and usage of equipment or reagents not included or recommended may lead to false results.

In case of doubt other validated methods for the detection of anti-erythrocytic antibodies should be included in the diagnostic evaluation.

5.2. Specific Characterization

REF	Diagn. sensitivity [%]	n=	Diagn. specificity [%]	n=
R_Ch(a)	100	22	100	10
R_CR1_2	100	21	100	17
R_CROM	100	8	100	15
R_Do(a)	100	9	100	8
R_Do(b)	100	9	100	12
R_Fy(a)	100	13	100	9
R_Fy(b)	100	3	100	11
R_grKba	100	15	100	10
R_In(b)	100	3	100	7
R_JMH	100	33	100	78
R_klkba	100	5	100	6
R_Lu(a)	100	7	100	12
R_Lu(a)_2	100	6	100	28
R_Lu(b)	100	19	99	156
R_Lu(b)_2	100	6	100	21
R_LW(a)	100	3	100	5
R_Rg(a)	100	15	100	12
R_Sc1	100	5	100	51
R_Xg(a)	100	3	100	7
R_YCAD	100	4	100	22
R_Yt(a)	100	6	100	6

6. References

1. Richtlinien zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Hämotherapie) gemäß §§ 12a und 18 des Transfusionsgesetztes (TFG) (Gesamtnovelle 2017 vom 17.02.2017). Bundesministerium der Justiz ISSN 0720-6100 Jahrgang 62.
2. Seltsam A., Blasczyk R. (2009). Curr Opin Hematol. 2009 Nov;16(6):473-9. doi: 10.1097/MOH.0b013e3283319a06.
3. Seltsam A., et al. (2014). Transfusion. Jul;54(7):1823-30. doi: 10.1111/trf.12553.

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⚠ ATTENTION!

Please check imusyn.de/IFU regularly for updates to this user manual.

Changes to the previous version are highlighted.

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